Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 4285–4294

TETRAHEDRON:
ASYMMETRY

(*R*)- and (*S*)-*N*-(Benzyloxycarbonyl)-3,4-epoxybutylamine. New 4-amino-2-hydroxybutyl synthons for the synthesis of hypusine reagents and (*R*)-6- and (*S*)-7-hydroxyspermidine

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Received 2 August 1999; accepted 10 October 1999

Abstract

The synthesis and application of the chiral reagents (*R*)- and (*S*)-*N*-(benzyloxycarbonyl)-3,4-epoxybutylamine is described for the first time. These 4-amino-2-hydroxybutyl synthons are successfully employed in the assembly of two hydroxylated triamines, (*R*)-6- and (*S*)-7-hydroxyspermidine, and a previously described hypusine reagent, (2*S*,9*R*)-11-[(benzyloxycarbonyl)amino]-7-(benzyloxycarbonyl)-2-[(9-fluorenylmethoxycarbonyl)amino]-9-(tetrahydropyran-2-yloxy)-7-azaundecanoic acid, useful for solution- and solid-phase peptide synthesis.

1. Introduction

The polyamines putrescine, spermidine, and spermine are found in all eukaryotes and are essential to cell growth. Although polyamine fragments have been identified in a host of siderophores,[1] spider toxins,[2] squalamine,[3] etc., very few natural products contain polyamines in which the methylene backbone itself is modified. The notable exceptions are hydroxyputrescine,[4] the triamines (*S*)-6- and 7-hydroxyspermidine, and hypusine. The latter three systems are the subject of this paper. Whereas 7-hydroxyspermidine occurs free in *Pseudomonas* species,[5,6] (*S*)-6-hydroxyspermidine is a component of several alkaloids isolated from marine microorganisms.[7,8] Hypusine, (2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid (**1**),[9] is an unusual amino acid which has attracted great interest in recent years because of its role in the replication of HIV.[10] It is formed by the post-translational modification of

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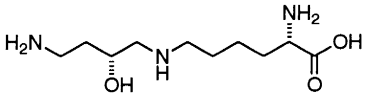
0957-4166/99/$ - see front matter
PII: S0957-4166(99)00474-7

eukaryotic initiation factor 5A (eIF-5A).[11] Lysine 50 of this protein is coupled with an aminobutyl fragment derived from spermidine followed by hydroxylation at C-9.[12] Thus, the hydroxylated polyamines are interesting from a synthetic standpoint because of the role they play in potential targets for various therapeutic strategies.



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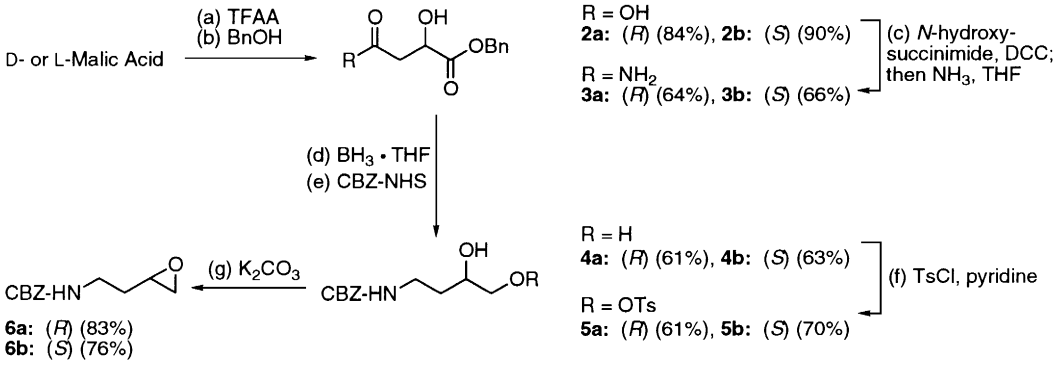
Although there are currently two synthetic approaches to the hydroxyspermidines, one leads to a mixture of 6- and 7-isomers;[6] the other yields racemic 6-hydroxyspermidine.[13] The synthons described below, (*R*)- and (*S*)-*N*-(benzyloxycarbonyl)-3,4-epoxybutylamine, allow access to enantiomerically pure 6- or 7-hydroxyspermidine. The same fragments also provide for an alternate, more direct, assembly of the hypusine reagent, (2*S*,9*R*)-11-[(benzyloxycarbonyl)amino]-7-(benzyloxycarbonyl)-2-[(9-fluorenylmethoxycarbonyl)amino]-9-(tetrahydropyran-2-yloxy)-7-azaundecanoic acid.[14,15] This reagent is a very powerful tool in synthesizing hypusine-containing peptides, which are key to expanding our understanding of how eIF-5A functions.

2. Results and discussion

The enantiomeric epoxides **6a** and **6b** were utilized in the construction of the triamines (*R*)-6- and (*S*)-7-hydroxyspermidine (**8** and **13**, respectively) and the hypusine reagent (**18**). These oxirane synthons were synthesized from tosylates **5a** and **5b**, which were in turn made from D- or L-malic acid, respectively (Scheme 1). Compounds **5a**,**b** were also prepared by Shiba et al. from the requisite malic acid by a different route and were converted using LiBr in acetone to (*R*)- or (*S*)-4-(benzyloxycarbonyl)amino-1-bromo-2-butanol. Next, each bromohydrin was condensed with the benzyl ester of N^{α}-(benzyloxycarbonyl)-L-lysine, tosylate salt; catalytic removal of the benzyl-derived protecting groups gave hypusine itself or its epimer in 20% optimized yield from tosylate **5a** or **5b**, respectively.[16]



Scheme 1.

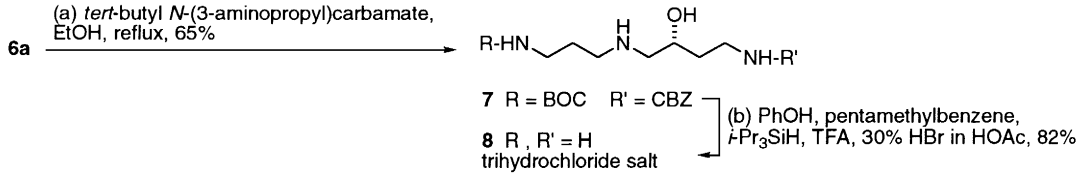
2.1. Epoxides *6a* and *6b*

The synthetic sequences began with the conversion of benzyl esters **2a**,**b**, which are readily available from D- or L- malic acid,[17] respectively, to their *N*-hydroxysuccinimide esters and subsequent reaction with ammonia to provide amide esters **3a**,**b** in 65% yield. Reduction of

3a,**b** using borane–tetrahydrofuran, followed by selective protection of the amino group with *N*-(benzyloxycarbonyloxy)succinimide (CBZ-NHS), yielded the diols **4a**,**b** (61% and 63%). Selective *O*-tosylation of **4a**,**b** in dichloromethane and pyridine afforded the mono-tosylates **5a**,**b**.[16] Finally, ring closure of **5a**,**b** with potassium carbonate (1.1 equiv., 1 h) in methanol gave the desired epoxides **6a**,**b** in 31% and 34% yield from **3a** and **3b**, respectively. Although oxiranes **6a**,**b** may have formed as intermediates in Shiba's base-promoted construction of the hypusine framework,[16] the first directed synthesis of these chiral epoxide synthons has been accomplished.

2.2. (R)-6-Hydroxyspermidine *8*

The synthesis of (*R*)-6-hydroxyspermidine (**8**, Scheme 2) was achieved by coupling oxirane **6a** with *tert*-butyl *N*-(3-aminopropyl)carbamate to provide the protected triamine **7** (65%). The CBZ and BOC protective groups were then removed in one step using HBr in acetic acid to give (*R*)-6-hydroxyspermidine (**8**) in 82% yield after conversion to its trihydrochloride salt, thus accomplishing the first synthesis of this polyamine in enantiomerically pure form.



Scheme 2.

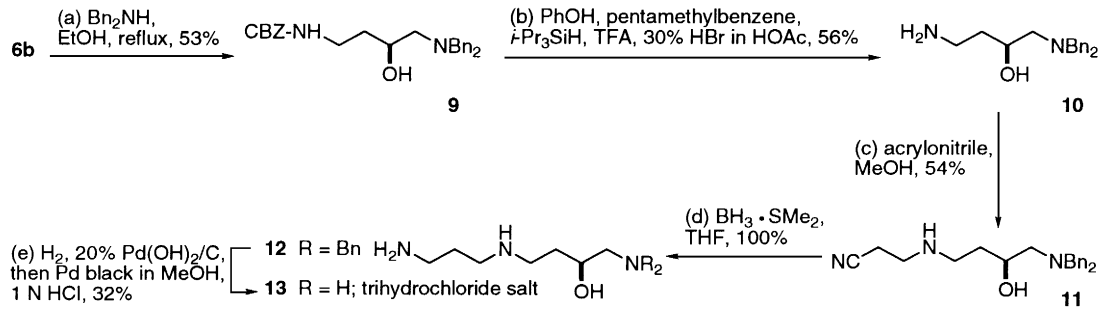
2.3. (S)-7-Hydroxyspermidine *13*

Similar methodology was also applied to the first synthesis of (*S*)-7-hydroxyspermidine (**13**, Scheme 3). Benzyl (*S*)-*N*-(3,4-epoxybutyl)carbamate (**6b**), derived from L-malic acid (Scheme 1), was treated with dibenzylamine (2 equiv.) in refluxing ethanol for 1 day, giving amino alcohol **9** in 53% yield. The benzyloxycarbonyl (CBZ) protecting group of **9** was cleaved by treatment with 30% HBr in HOAc/TFA (phenol/pentamethylbenzene/triisopropylsilane)[15] at rt for 1 h to furnish (*S*)-N^1,N^1-dibenzyl-2-hydroxyputrescine (**10**) in 56% yield. Exposure of **10** to an equivalent of acrylonitrile in methanol for 1 d at rt led to mono-cyanoethylated[18] diamine **11** in 54% yield, thus completing the polyamine skeleton. An attempt to catalytically cleave the benzyls and saturate the nitrile of **11** (Pd black/formic acid) failed to give the final polyamine because of incomplete reaction; thus, a stepwise approach was employed. The cyano moiety of **11** was selectively reduced using borane–dimethyl sulfide complex in THF (15 min, reflux),[19] generating (*S*)-N^8,N^8-dibenzyl-7-hydroxyspermidine (**12**) in quantitative yield.

The *gem*-dibenzyl blocking groups of **12** were not cleanly removed by dissolving metal reduction (Na/NH_3/THF/–78°C) or prolonged hydrogenolysis over 10% Pd–C in methanolic HCl. Successful debenzylation required exposure to two different catalysts. First, hydrogenation of **12** over Pearlman's catalyst [20% $Pd(OH)_2$–C/MeOH/3 days],[20,21] followed by reduction over palladium black (1N HCl/MeOH), provided the target **13** as its trihydrochloride salt (32%).

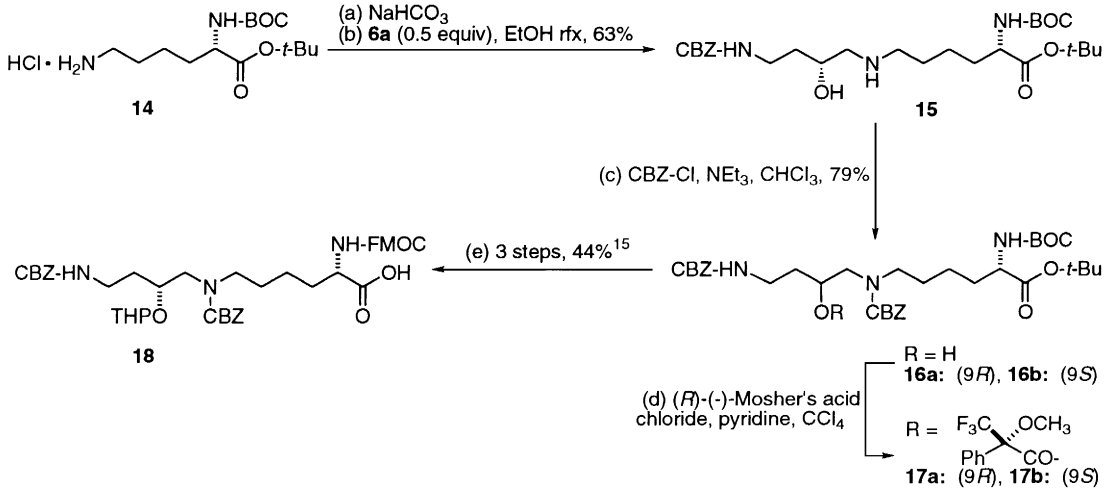
2.4. Extension to the hypusine reagent *18*

The application of the epoxide **6a** was extended to the synthesis of **18**, an adaptable hypusine reagent (Scheme 4).[14,15] In this reagent all three nitrogens and the 9-hydroxyl are protected while the carboxyl



Scheme 3.

group remains free for peptide coupling. The α-amino nitrogen is protected as the 9-fluorenylmethyl carbamate (FMOC); the two remaining amines are masked as carbobenzyloxy (CBZ) moieties. The 9-hydroxyl is protected as a tetrahydropyranyl ether (THP). For further peptide coupling, the α-amino group can be cleanly unmasked in the presence of the other protecting groups. Thus, **18** allows for the assembly of a variety of hypusine-containing peptides. Two syntheses of this reagent have been published from our laboratories.[14,15] In both, the chiral 4-amino-2-hydroxybutyl group was attached at the ε-nitrogen of a lysine derivative employing epichlorohydrin, followed by displacement of the halide with cyanide. This part of the synthesis does not lend itself to scaleup. In the first route,[14] the internal nitrogen of the reagent was protected by a benzyl group. Cyanide replaced the halide of the intermediate 3-amino chlorohydrin in the following step; racemization can occur if the reaction conditions are not carefully controlled. This was thought to occur by formation of an azetidinium intermediate, ring opening of which would lead to epimerization. In the second route,[15] the secondary nitrogen was protected by a CBZ, vitiating the nucleophilicity of the nitrogen's non-bonding pair and preventing azetidinium formation.



Scheme 4.

In our new synthetic approach, the *tert*-butyl ester of N^{α}-BOC-L-lysine, hydrochloride salt (**14**),[15] was treated with sodium bicarbonate solution (Scheme 4) and then heated for 1 day with the (*R*)-epoxide **6a** (0.5 equiv.) in ethanol producing amino alcohol **15** (56% yield), which possesses the hypusine skeleton. The assembly of orthogonally-protected hypusine **15** from tosylate **5a** was accomplished in 52% yield for the two steps, which is more than twice as efficient as Shiba's production of hypusine via its benzyl

group-protected derivative, also from intermediate **5a**.[16] Protection of the secondary amine of **15** was effected with benzyl chloroformate (NEt_3, $CHCl_3$) to furnish **16a** in 79% yield.[14,15] The final three steps, i.e., removal of the *tert*-butyl protective groups of **16a** with TFA/triethylsilane and protection of the 9-hydroxyl and the α-amino functions as the THP ether and FMOC derivative, respectively, followed our previously described procedures[14,15] and provided the (9*R*)-hypusine reagent **18** in good yield (44%, Scheme 4). The (9*S*)-diastereomer of **18** could be readily prepared by using (*S*)-epoxide reagent **6b** in Scheme 4.

To confirm the stereochemical integrity at the C-9 position of **18**, alcohol **16a** (Scheme 4) was converted to its Mosher ester[22] derivative **17a** (88%). We also synthesized Mosher ester **17b** from **16b** (70%), which was produced by a different method.[15] Resonances at −71.23 and −71.37 ppm were observed in the ^{19}F NMR spectrum of **17b** at 25°C. However, these peaks had coalesced at 45°C to a sharp signal at −71.41 ppm, most likely indicating the presence of rotamers. The peak at −71.41 ppm was distinct from the corresponding resonance of **17a** at −71.74 ppm. Since we were not able to observe the −71.41 ppm resonance of **17b** in the spectrum of **17a** nor the −71.74 ppm signal of **17a** in the NMR of **17b**, it is concluded that the synthesis of reagent **6a**, its coupling with the protected lysine **14** to produce **15**, and the subsequent steps in Scheme 4 had not been accompanied by racemization of the secondary alcohol at C-9.

3. Conclusions

In summary, new enantiomeric epoxide reagents lead efficiently to chain functionalized polyamines in which a four-carbon unit has a chiral secondary alcohol. The epoxide synthons also provide stereochemically controlled access to a versatile hypusine reagent, which is in turn a highly useful synthon in accessing the eIF-5A pentapeptide sequence and eIF-5A mimics.

4. Experimental

Reagents were purchased from Aldrich (Milwaukee, WI), Fluka (Milwaukee, WI), or Sigma (St. Louis, MO) Chemical Co. and were used without purification. L- and D-Malic acids were obtained from Aldrich in 99% and 98% e.e., respectively. Fisher Optima grade solvents were routinely used. THF was distilled from sodium metal and benzophenone, and CH_3OH and EtOH were also distilled. Organic extracts were dried over Na_2SO_4, and silica gel 32–60 (40 μm 'flash') from Selecto, Inc. (Kennesaw, GA) was used for flash column chromatography unless otherwise indicated. Melting points are uncorrected. Proton NMR spectra were run at 300 MHz in deuterated organic solvents or in D_2O with chemical shifts in parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt, respectively. Coupling constants (*J*) are in hertz. High resolution mass spectra were run in a 3-nitrobenzyl alcohol matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

*4.1. General procedure for generation of malic acid derivatives **3a**,**b***

*4.1.1. Benzyl (*R*)-4-amino-2-hydroxy-4-oxobutanoate **3a***

N-Hydroxysuccinimide (287 mg, 2.49 mmol) and DCC (514 mg, 2.49 mmol) were added to a solution of **2a**[17] (508 mg, 2.27 mmol) in CH_2Cl_2 (25 mL) under N_2. After 90 min, the mixture was filtered, the DCC was washed with CH_2Cl_2, and the solvent was removed in vacuo. The residue was dissolved in

dry THF (70 mL), cooled to 0°C, and ammonia gas was introduced into the solution over 20 min. The reaction mixture was stirred for 20 min at 0°C and then concentrated to dryness. Flash chromatography of the residue (12:1 $CHCl_3$:CH_3OH) afforded 324 mg (64%) of **3a** as a colorless solid, mp 77–79°C. ^{1}H NMR ($CDCl_3$): 2.66 (dd, 1H, *J*=15.6, 7.0), 2.77 (dd, 1H, *J*=15.6, 3.9), 3.80 (br s, 1H), 4.53 (dd, 1H, *J*=7.0, 3.9), 5.23 (s, 2H), 5.80 (br s, 1H), 6.00 (br s, 1H), 7.36 (m, 5H). $[\alpha]_D^{23}$ +18.6 (*c* 2.0, CH_3OH) [lit. *ent*-**3a**:[23] $[\alpha]_D^{25}$ –17.1 (*c* 2, CH_3OH)]. Anal. calcd: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.20; H, 5.80; N, 6.28.

*4.1.2. Benzyl (S)-4-amino-2-hydroxy-4-oxobutanoate **3b***

Compound **2b**[17] (15.2 g, 67.7 mmol) was reacted by the method of **3a** to give 9.95 g (66%) of **3b** as a colorless solid. ^{1}H NMR ($CDCl_3$): 2.66 (dd, 1H, *J*=15.6, 7.3), 2.77 (dd, 1H, *J*=15.6, 3.7), 3.70 (d, 1H, *J*=4.8), 4.53 (m, 1H), 5.23 (s, 2H), 5.55 (br m, 1H), 5.91 (br m, 1H), 7.36 (m, 5H). A sample was recrystallized from cyclohexane/EtOAc, mp 79.2–80.5°C (lit. **3b**:[23] 78–80°C). Anal. calcd: C, 59.19; H, 5.87; N, 6.27. Found: C, 58.98; H, 5.82; N, 6.41.

*4.2. General procedure for reduction to alcohols **4a**,**b***

*4.2.1. (R)-4-(Benzyloxycarbonyl)amino-1,2-butanediol **4a***

A 1 M solution of BH_3 in THF (100 mL) was added to a stirred solution of **3a** (4.35 g, 19.5 mmol) in THF (100 mL) at rt under N_2. After the mixture was stirred for 17 h, 4N aqueous HCl was slowly added until pH 4 was achieved. The reaction mixture was stirred at rt for 4 h. The bulk of the THF was removed by rotary evaporation; the residue was neutralized with 1N NaOH and concentrated to dryness under high vacuum.[24] Water (60 mL) and Et_2O (100 mL) were added to the residue. *N*-(Benzyloxycarbonyloxy)succinimide (5.34 g, 21.4 mmol) and $NaHCO_3$ (1.64 g, 19.5 mmol) were added at 0°C, and the mixture was stirred at rt for 18 h. The layers were separated, and the aqueous layer was further extracted with EtOAc (3×100 mL). The organic layers were washed with $NaHCO_3$ (50 mL), the $NaHCO_3$ layer was re-extracted with EtOAc (2×50 mL), and the solvent was removed in vacuo. Flash chromatography (12:1 $CHCl_3$:CH_3OH) gave 2.82 g (61%) of **4a** as a colorless solid, mp 64–65°C (lit.[16] 67–68°C). ^{1}H NMR (CD_3OD): 1.52 (m, 1H), 1.71 (m, 1H), 3.25 (m, 2H), 3.45 (m, 2H), 3.63 (m, 1H), 5.06 (s, 2H), 7.31 (m, 5H). $[\alpha]_D^{22}$ +6.3 (*c* 1.97, $CHCl_3$) [lit.[16] $[\alpha]_D^{22}$ +5.8 (*c* 1.9, $CHCl_3$)].

*4.2.2. (S)-4-(Benzyloxycarbonyl)amino-1,2-butanediol **4b***

Compound **3b** (10.60 g, 47.50 mmol) was treated as was **3a** to give 7.18 g (63%) of **4b** as a colorless solid. $[\alpha]_D^{21}$ –6.0 (*c* 1.9, $CHCl_3$) [lit.[16] $[\alpha]_D^{25}$ –6.0 (*c* 2.2, $CHCl_3$)].

*4.3. General procedure for epoxide reagents **6a**,**b***

*4.3.1. (R)-N-(Benzyloxycarbonyl)-3,4-epoxybutylamine **6a***

Potassium carbonate (151 mg, 1.09 mmol) was added to a solution of **5a**[16] (391 mg, 0.99 mmol) in CH_3OH (30 mL). The mixture was stirred at rt under N_2 for 50 min, then poured into a mixture of H_2O (50 mL) and CH_2Cl_2 (40 mL). The layers were separated, the aqueous layer was extracted with CH_2Cl_2 (4×30 mL), and the solvent was removed in vacuo. Flash chromatography (80:1 $CHCl_3$:CH_3OH) afforded 182 mg (83%) of **6a** as a colorless oil. ^{1}H NMR (CD_3OD): 1.63 (m, 1H), 1.76 (m, 1H), 2.47 (m, 1H), 2.71 (m, 1H), 2.95 (m, 1H), 3.27 (m, 2H), 5.07 (s, 2H), 7.33 (m, 5H). $[\alpha]_D^{22}$ +21.4 (*c* 1.00, $CHCl_3$). Anal. calcd: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.15; H, 6.80; N, 6.33.

*4.3.2. (S)-N-(Benzyloxycarbonyl)-3,4-epoxybutylamine **6b***

In the same manner as for **6a**, **5b**[16] (8.74 g, 22.2 mmol) was converted to 3.72 g (76%) of **6b** as a colorless oil. $[\alpha]_D^{22}$ –21.8 (*c* 1.07, $CHCl_3$). Anal. calcd: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.07; H, 6.89; N, 6.27.

*4.4. (R)-N^1-(tert-Butoxycarbonyl)-N^8-(benzyloxycarbonyl)-6-hydroxyspermidine **7***

A solution of **6a** (152 mg, 0.69 mmol) and *tert*-butyl *N*-(3-aminopropyl)carbamate (239 mg, 1.37 mmol) in EtOH (20 mL) was heated to reflux for 18 h under Ar. The reaction mixture was concentrated in vacuo and purified by flash chromatography (10%, then 50% $CH_3OH/CHCl_3$) to give 175 mg (65%) of **7** as a white solid, mp 90–92°C. ^{1}H NMR (CD_3OD): 1.43 (s, 9H), 1.50–1.77 (m, 4H), 2.50–2.78 (m, 4H), 3.09 (t, 2H, *J*=6.6), 3.25 (t, 2H, *J*=7.0), 3.74 (m, 1H), 5.06 (s, 2H), 7.23–7.45 (m, 5H). $[\alpha]_D^{23}$ +2.8 (*c* 1.05, CH_3OH). HRMS *m/z* calcd for $C_{20}H_{34}N_3O_5$: 396.2498; found: 396.2523.

*4.5. (R)-6-Hydroxyspermidine trihydrochloride **8***

HBr in acetic acid (30%, 400 μL) was added to a solution of **7** (155 mg, 0.39 mmol), phenol (500 mg), pentamethylbenzene (500 mg), and triisopropylsilane (200 μL) in TFA (5 mL) at 0°C under Ar. The ice bath was removed after 10 min, and the reaction mixture was stirred at rt for an additional 50 min and concentrated in vacuo. The residue was dissolved in 10% acetic acid (20 mL) and extracted with *tert*-butyl methyl ether (3×25 mL). The aqueous layer was concentrated and dried in vacuo. The residue was dissolved in H_2O (5 mL) and the pH was adjusted to 7 with saturated $NaHCO_3$. The solution was concentrated in vacuo and purified by flash chromatography (Kieselgel 60, 70–230 mesh, Merck), eluting with freshly prepared 1:2:1 CH_2Cl_2:CH_3OH:conc. NH_4OH. The oil was dissolved in CH_3OH (4 mL) and filtered through a syringe filter (Whatman PVDF 0.2 μm). The filtrate was concentrated in vacuo, dissolved in H_2O (2 mL), acidified with 1N HCl (4 mL), and concentrated. The resulting solid was dissolved in CH_3OH (5 mL) and precipitated with EtOAc (9 mL) to give 87 mg (82%) of **8** as a white, hygroscopic powder, mp 192–193°C. ^{1}H NMR (D_2O): 1.76–2.04 (m, 2H), 2.04–2.24 (m, 2H), 3.04–3.38 (m, 8H), 4.08 (tt, 1H, *J*=9.5, 3.2). ^{13}C NMR (75 MHz, D_2O, dioxane=67.19 ppm): 24.20, 31.96, 37.03, 37.18, 45.28, 52.88, 65.32. $[\alpha]_D^{24}$ –7.1 (*c* 1.02, H_2O). HRMS *m/z* calcd for $C_7H_{20}N_3O$: 162.1606; found: 162.1604. Anal. calcd: C, 31.07; H, 8.19; Cl, 39.30; N, 15.53. Found: C, 31.01; H, 8.07; Cl, 39.14; N, 15.32.

*4.6. (S)-4-[(Benzyloxycarbonyl)amino]-1-(dibenzylamino)-2-butanol **9***

Dibenzylamine (1.02 g, 5.17 mmol) and **6b** (513 mg, 2.32 mmol) were heated at reflux in EtOH (20 mL) for 20 h under N_2. The solution was concentrated to dryness, and the residue was purified twice by flash chromatography (2% CH_3OH/CH_2Cl_2) affording 520 mg (53%) of **9** as a colorless oil. ^{1}H NMR (CD_3OD): 1.31 (m, 1H), 1.76 (m, 1H), 2.43 (m, 2H), 3.14 (m, 2H), 3.57 (s, 4H), 3.72 (m, 1H), 5.05 (s, 2H), 7.17–7.37 (m, 15H). ^{13}C NMR (75 MHz, CD_3OD, ref. solvent 49.0 ppm): 36.0, 38.3, 38.7, 60.0, 60.8, 67.3, 67.6, 128.1, 128.7, 128.9, 129.3, 129.4, 130.2, 138.4, 140.5, 158.8. Anal. calcd: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.32; H, 7.15; N, 6.65.

4.7. (S)-4-Amino-1-(dibenzylamino)-2-butanol **10**

HBr in HOAc (30%, 0.20 mL) was added to a solution of **9** (520 mg, 1.24 mmol), phenol (250 mg), pentamethylbenzene (250 mg), and triisopropylsilane (0.10 mL) in TFA (5.0 mL) at 0°C under N_2. The solution was stirred for 5 min at 0°C, then for 30 min at rt. Solvent removal gave a crude oil, to which was added 90% aqueous HOAc (20 mL) at 0°C followed by extraction with *tert*-butyl methyl ether (3×50 mL). Concentration of the aqueous layer in vacuo and purification by flash chromatography (3% concentrated NH_4OH/CH_3OH) gave 197 mg (56%) of **10** as a colorless oil. ^{1}H NMR (CD_3OD): 1.37 (m, 1H), 1.71 (m, 1H), 2.44 (d, 2H, *J*=6.9), 2.69 (m, 2H), 3.60 (s, 4H), 3.78 (m, 1H), 7.19–7.38 (m, 10H). HRMS *m/z* calcd for $C_{18}H_{25}N_2O$: 285.1967; found: 285.2024.

4.8. (S)-8-(Dibenzylamino)-7-hydroxy-4-azaoctanenitrile **11**

Acrylonitrile (37.6 mg, 0.709 mmol) was slowly added to **10** (197 mg, 0.693 mmol) in CH_3OH (1.0 mL) under N_2; the reaction solution was gradually warmed to rt and stirred for a total of 21 h. The solvent was evaporated, and the residue was purified by flash chromatography (4% CH_3OH/CH_2Cl_2) to give 126 mg (54%) of **11** as a colorless oil. ^{1}H NMR (CD_3OD): 1.31–1.46 (m, 1H), 1.68–1.81 (m, 1H), 2.45 (d, 2H, *J*=6.5), 2.51–2.62 (m, 4H), 2.78 (t, 2H, *J*=6.8), 3.59 (dd, 4H, *J*=17.7, 13.3), 3.79 (m, 1H), 7.19–7.38 (m, 10H). HRMS *m/z* calcd for $C_{21}H_{28}N_3O$: 338.2232; found: 338.2195.

4.9. (S)-N^8,N^8-Dibenzyl-7-hydroxyspermidine **12**

Borane–methyl sulfide complex in THF (2 M, 1.8 mL, 3.6 mmol) was added by syringe to **11** (125.5 mg, 0.373 mmol) under N_2. The reaction was stirred at rt for 15 min, then refluxed for an additional 15 min. After cooling, 6 M HCl (2.5 mL) was added and the solution was stirred for 40 min. The solvent was evaporated and the crude product was redissolved in 1N NaOH (15 mL), then extracted with $CHCl_3$ (3×25 mL). The organic layer was washed with H_2O (20 mL), then concentrated to give 127 mg (100%) of **12** as a colorless oil. ^{1}H NMR (CD_3OD): 1.40 (m, 1H), 1.63 (m, 2H), 1.67–1.81 (m, 1H), 2.45 (d, 2H, *J*=6.4), 2.48–2.62 (m, 4H), 2.66 (t, 2H, *J*=7.1), 3.59 (dd, 4H, *J*=15.4, 13.5), 3.77 (m, 1H), 7.19–7.38 (m, 10H). HRMS *m/z* calcd for $C_{21}H_{32}N_3O$: 342.2545; found: 342.2545.

4.10. (S)-7-Hydroxyspermidine trihydrochloride **13**

Compound **12** (127.3 mg, 0.373 mmol) was stirred with 20% $Pd(OH)_2$–C (62 mg) in CH_3OH (7.5 mL) under hydrogen for 3 days at 1 atm. The reaction mixture was filtered through Celite, evaporated to dryness, and hydrogenated again at ambient pressure and temperature for 24 h over Pd black (32 mg) in CH_3OH (12 mL) and 1N HCl (1.6 mL). After filtration through Celite, the solvent was evaporated. The crude product was treated with 1N HCl (10 mL) and evaporated to give a white crystalline powder. Recrystallization from 25% Et_2O/CH_3OH (15 mL) gave 32 mg (32%) of **13** as fine white crystals, mp 203–206°C. ^{1}H NMR (D_2O): 1.78–2.17 (m, 4H), 2.97 (dd, 1H, *J*=13.3, 9.5), 3.07–3.36 (m, 7H), 4.00 (tt, 1H, *J*=9.5, 3.3). ^{13}C NMR APT (75.4 MHz, D_2O, dioxane=67.19 ppm, APT inverse peaks underlined): 24.4, 30.8, 37.2, 44.9, 45.26, 45.31, <u>66.0</u>. $[\alpha]_D^{24}$ –0.5 (*c* 1.00, 1N HCl), +1.1 (*c* 1.00, CH_3OH). Anal. calcd: C, 31.07; H, 8.19; Cl, 39.30; N, 15.53. Found: C, 31.34; H, 8.36; Cl, 39.55; N, 15.24.

4.11. tert-*Butyl (2*S,*9*R*)-11-[(benzyloxycarbonyl)amino]-2-[(*tert-*butoxycarbonyl)amino]-9-hydroxy-7-azaundecanoate* ***15***

A solution of **14**[15] (171 mg, 0.51 mmol) in $CHCl_3$ (20 mL) was extracted with saturated $NaHCO_3$ (2×20 mL). The combined aqueous layers were extracted with $CHCl_3$ (2×20 mL). The combined organic layers were washed with saturated NaCl (20 mL) and concentrated to give the primary amine (136 mg, 89%) as a colorless oil, a portion of which (52 mg, 172 mmol) was heated at reflux for 23 h under Ar with **6a** (19 mg, 86 mmol) in EtOH (10 mL). The solvent was removed in vacuo and the residue was purified by flash chromatography (2%, then 20% $CH_3OH/CHCl_3$) to give 29 mg (63%) of **15** as a colorless oil. ^{1}H NMR (CD_3OD): 1.35–1.82 (m, 8H), 1.44 (s, 9H), 1.46 (s, 9H), 2.48–2.74 (m, 4H), 3.25 (t, 2H, *J*=6.8), 3.74 (m, 1H), 3.94 (dd, 1H, *J*=8.6, 5.1), 5.06 (s, 2H), 7.24–7.40 (m, 5H). $[\alpha]_D^{22}$ –12.3 (*c* 1.00, CH_3OH). HRMS *m/z* calcd for $C_{27}H_{46}N_3O_7$: 524.3336; found: 524.3261.

4.12. tert-*Butyl (2*S,*9*R*)-11-[(benzyloxycarbonyl)amino]-2-[(*tert-*butoxycarbonyl)amino]-9-hydroxy-7-(benzyloxycarbonyl)-7-azaundecanoate* ***16a***

Benzyl chloroformate (10 μL, 70 μmol) and triethylamine (15 μL, 110 μmol) were added to a solution of **15** (27 mg, 52 μmol) in $CHCl_3$ (2 mL) at 0°C under Ar. The reaction mixture was stirred at rt for 1.5 h, diluted with $CHCl_3$ (20 mL), and successively washed with 1N HCl (10 mL), H_2O (10 mL), and saturated NaCl (10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (50% hexane/EtOAc) gave 27 mg (79%) of **16a** as a colorless oil. ^{1}H NMR (CD_3OD): 1.25–1.80 (m, 8H), 1.43 (s, 9H), 1.44 (s br, 9H), 3.08–3.48 (m, 6H), 3.82 (m, 1H), 3.92 (dd, 1H, *J*=8.0, 5.3), 5.06 (s, 2H), 5.11 (s, 2H), 7.24–7.39 (m, 10H). $[\alpha]_D^{22}$ –12.5 (*c* 1.00, CH_3OH).

4.13. General procedure for derivatization of ***16a,b*** *to Mosher esters* ***17a,b***

4.13.1. tert-*Butyl (2*S,*9*R*)-11-[(benzyloxycarbonyl)amino]-2-[*tert-*(butoxycarbonyl)amino]-7-(benzyloxycarbonyl)-9-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetoxy]-7-azaundecanoate* ***17a***

The reaction was carried out in an oven-dried 5×175 mm NMR tube, fitted with a rubber septum, under Ar. The reagents were injected via syringe in the following order: anhydrous pyridine (300 μL), (*R*)-(−)-Mosher's acid chloride (12 μL, 57 μmol), anhydrous CCl_4 (200 μL), and a solution of **16a** (22 mg, 33 μmol) in anhydrous CCl_4 (500 μL). The reaction mixture was shaken and allowed to stand at rt for 18 h. The reaction mixture was then taken up in $CHCl_3$ (20 mL) and washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was concentrated in vacuo. The residue was purified by flash chromatography (25% EtOAc/hexane) to give 26 mg (88%) of **17a** as a colorless oil. ^{1}H NMR ($CDCl_3$, 45°C): 1.10–1.88 (m, 8H), 1.44 (s, 9H), 1.45 (s, 9H), 2.80 (m, 1H), 2.95–3.60 (m, 4H), 3.28 (dd, 1H, *J*=14.6, 7.5), 3.50 (s, 3H), 4.09 (m, 1H), 4.80–5.38 (m, 7H), 7.25–7.55 (m, 15H). ^{19}F NMR (282 MHz, $CDCl_3$, $CFCl_3$ as internal standard, 45°C): −71.74. $[\alpha]_D^{21}$ −12.6 (*c* 1.00, $CHCl_3$). HRMS *m/z* calcd for $C_{45}H_{59}F_3N_3O_{11}$: 874.4102; found: 874.4100.

4.13.2. tert-*Butyl (2*S,*9*S*)-11-[(benzyloxycarbonyl)amino]-2-[*tert-*(butoxycarbonyl)amino]-7-(benzyloxycarbonyl)-9-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetoxy]-7-azaundecanoate* ***17b***

According to the method described for the preparation of **17a**, **16b**[15] (14 mg, 21 μmol) was reacted with (*R*)-(–)-Mosher's acid chloride (10 μL, 54 μmol) to give 13 mg (70%) of **17b** as a colorless oil. ^{1}H NMR ($CDCl_3$, 45°C): 1.15–1.90 (m, 8H), 1.44 (s, 9H), 1.44 (s, 9H), 2.92–3.60 (m, 6H), 3.44 (s, 3H), 4.10 (m, 1H), 4.80–5.40 (m, 7H), 7.22–7.60 (m, 15H). ^{19}F NMR (282 MHz, $CDCl_3$, $CFCl_3$ as internal

standard, 45°C): −71.41. $[\alpha]_D^{22}$ −9.7 (*c* 0.98, $CHCl_3$). HRMS *m/z* calcd for $C_{45}H_{59}F_3N_3O_{11}$: 874.4102; found: 874.4173.

Acknowledgements

This work was supported by SunPharm Corporation, Ponte Vedra Beach, FL. The work of the Spectroscopy Services Group, Department of Chemistry, University of Florida, for HRMS analyses is appreciated. We also thank Dr. E. Eiler-Hughes for her editorial comments.

References

1. Bergeron, R. J.; McManis, J. S. Synthesis of catecholamide and hydroxamate siderophores. In *CRC Handbook of Microbial Iron Chelates*; Winkelmann, G., Ed.; CRC: Boca Raton, 1991; pp. 271–307.
2. Moya, E.; Blagbrough, I. S. *Tetrahedron Lett.* **1994,** *35*, 2061–2062.
3. Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N., Jr.; McCrimmon, D.; Zasloff, M. *Proc. Natl. Acad. Sci. USA* **1993,** *90*, 1354–1358.
4. Kullnig, R. K.; Rosano, C. L.; Coulter, M. E.; Hurwitz, C. *J. Biol. Chem.* **1973,** *248*, 2487–2488.
5. Hamana, K.; Matsuzaki, S. *FEMS Microbiol. Lett.* **1990,** *70*, 353–356.
6. Rosano, C. L.; Hurwitz, C.; Bunce, S. C. *J. Bacteriol.* **1978,** *135*, 805–808.
7. Palagiano, E.; DeMarino, S.; Minale, L.; Riccio, R.; Zollo, F.; Iorizzi, M.; Carré, J. B.; Debitus, C.; Lucarain, L.; Provost, J. *Tetrahedron* **1995,** *51*, 3675–3682.
8. Jares-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991,** *56*, 5712–5715.
9. Shiba, T.; Mizote, H.; Kaneko, T.; Nakajima, T.; Kakimoto, Y.; Sano, I. *Biochim. Biophys. Acta* **1971,** *244*, 523–531.
10. Ruhl, M.; Himmelspach, M.; Bahr, G. M.; Hammerschmid, F.; Jaksche, H.; Wolff, B.; Aschauer, H.; Farrington, G. K.; Probst, H.; Bevec, D.; Hauber, J. *J. Cell Biol.* **1993,** *123*, 1309–1320.
11. Park, M. H.; Cooper, H. L.; Folk, J. E. *J. Biol. Chem.* **1982,** *257*, 7217–7222.
12. Park, M. H.; Wolff, E. C.; Folk, J. E. *Trends Biochem. Sci.* **1993,** *18*, 475–479.
13. Ponasik, J. A.; Ganem, B. *Tetrahedron Lett.* **1995,** *36*, 9109–9112.
14. Bergeron, R. J.; Ludin, C.; Müller, R.; Smith, R. E.; Phanstiel IV, O. *J. Org. Chem.* **1997,** *62*, 3285–3290.
15. Bergeron, R. J.; Weimar, W. R.; Müller, R.; Zimmerman, C. O.; McCosar, B. H.; Yao, H.; Smith, R. E. *J. Med. Chem.* **1998,** *41*, 3888–3900.
16. Shiba, T.; Akiyama, H.; Umeda, I.; Okada, S.; Wakamiya, T. *Bull. Chem. Soc. Jpn.* **1982,** *55*, 899–903.
17. Cammas, S.; Renard, I.; Boutault, K.; Guérin, P. *Tetrahedron: Asymmetry* **1993,** *4*, 1925–1930.
18. Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1988,** *53*, 3108–3111.
19. Brown, H. C.; Choi, Y. M.; Narasimhan, S. *Synthesis* **1981**, 605–606.
20. Pearlman, W. M. *Tetrahedron Lett.* **1967,** *17*, 1663–1664.
21. Yoshida, K.; Nakajima, S.; Wakamatsu, T.; Ban, Y.; Shibasaki, M. *Heterocycles* **1988,** *27*, 1167–1168.
22. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973,** *95*, 512–519.
23. Roy, J.; Gazis, D.; Shakman, R.; Schwartz, I. L. *Int. J. Pept. Protein Res.* **1982,** *20*, 35–42.
24. Wang, G.; Hollingsworth, R. I. *J. Org. Chem.* **1999,** *64*, 1036–1038.